REMARKS

i. Status of the claims

Claims 1-3, 6-8, 12-17, and 53 are pending.

Claims 4, 5, and 18-52 have been canceled previously.

No claim has been added.

Claim 2 has been canceled because claim 1 has been amended to recite "positively-charged," which is the subject matter of original claim 2.

Claims 1, 7, 8, 12, 17, and 53 have been amended for the reasons that follow.

(a) *Claim 1*

Claim 1 is amended solely to indicate that the antigen is positively-charged.

(b) *Claim 7*

Claim 7 is amended to delete multiple dependencies and to depend from claim 1.

(c) *Claim 8*

Claim 8 is amended to replace "peptide" with "antigen," thereby ensuring correct antecedent basis with the preceding claims from which it depends.

(d) *Claim 12*

Applicants have replaced "adjuvant" recited in claim 12 with "organic complex" to ensure there is correct antecedent basis with the elements of claim 1 from which it depends.

(e) *Claim 17*

Applicants have amended claim 17 to clarify that the complex that induces a cytotoxic T-lymphocytic response is the "immunogenic" complex.

(f) *Claim 53*

Claim 53 is amended to relate that (i) the organic complex comprises saponin and sterol and (ii) the organic complex is modified to increase the degree of its negative charge and/or the antigen is modified to increase the degree of its positive charge. This amendment is fully supported, as evidenced, for instance, by original claims 6 and 7.

ii. Since Berglindh's lipid aggregate does not comprise a sterol-saponin complex, the reference does not anticipate claim 1, which recites such a complex

Claims 1-3, 12-17, and 53 are rejected under 35 U.S.C. § 102(a) as allegedly anticipated by WO 98/22135 ("Berglindh"), as evidenced by Cox *et al.*, *Vaccine*, 15(3), pp. 248-256, 1997. According to the Examiner, "Berglindh *et al.* anticipate a pharmaceutical formulation comprising a negatively charged lipid complex and an antigenic peptide that are electrostatically associated." Office Action at page 3.

The present immunogenic complex comprises a negatively-charged "organic complex" that is electrostatically associated with a positively-charged antigen. One benefit of this unit is its ability to facilitate "co-delivery of these molecules to the immune system." Application at page 9, lines 1-6.

Beyond this unexpected result, the immunogenic complex of claim 1 is structurally distinguishable over the teachings of Berglindh. In particular, the "organic complex" component requires "an entity of two or more different interacting chemical components" (emphasis added), namely, sterol and saponin. *Id.*, lines 14-15.

Berglindh arguably suggests the possibility of "combining" negatively charged lipids with "one or more additives," such as cholesterol or saponin. See page 11, lines 8-14. The reference does implicate any particular arrangement for those additives; certainly, no specific relationship between saponin and sterol.

Thus, Berglindh does not suggest a "complex" of saponin and sterol, as presently recited. To the contrary, the skilled artisan would have expected the presence of negatively-

charged saponin to disrupt the tertiary ("cochleate") structure that is an essential feature of the lipid aggregate obtained via Berglindh's manufacturing protocol.

According to Berglindh, at page 3, lines 23-26, cochleates are "large, cylindrical structures with lipid multilayers in a spiral configuration" produced by mixtures of phosphatidylserine and calcium ions in water. Berglindh's protocol likewise employs phosphatidylserine and calcium ions. As detailed on page 11, line 19 through page 12, line 9, Berglindh's protocol entails production of micelles by the addition of a surfactant-suspended antigen to a pre-formed, negatively-charged lipid film. The surfactant then is exchanged for Ca^{2+} ion, the presence of which, due to interaction with negative charges on the lipid film, results in fusion of the acidic lipid-antigen "micelles" or vesicles to produce cochleates or "lipid aggregates." See also K. Arnold, "Cation-Induced Vesicle Fusion Modulated by Polymers and Proteins," *in* 1 HANDBOOK OF BIOLOGICAL PHYSICS Ch. 19 (1995), at pages 909 and 910 (Exhibit 1).

Any attempt to incorporate negatively-charged saponin at any point in this protocol would disrupt the Ca^{2+}/lipid interaction and, hence, the overall formation of the cochleate/aggregate, contrary to Berglindh's stated purpose. Thus, the skilled person would understand, from Berglindh's passing mention of saponin, that the latter could only be added to a pre-formed cochleate/aggregate structure. In this scenario, however, the saponin would not be "complexed" predictably with any other component; rather, it likely would adhere to the outer surface of the cochleate/aggregate, in sharp contrast to the presently claimed invention. Moreover, the Berglindh protocol has no place for the addition of sterol, somehow to provide these components in an interacting unit ("complex"), as recited in Applicants' claims.

For at least these reasons, the immunogenic complex of claim 1 is not anticipated by Berglindh. Accordingly, Applicants respectfully request the withdrawal of this rejection.

iii. Popescu fails to teach each and every element of claim 53 because it likewise does not disclose modifying an organic complex or an antigen to increase electrostatic charge

Claim 53 is rejected under 35 U.S.C. § 102(e) as allegedly anticipated by U.S. Patent No. 5,897,873 ("Popescu") and under section 102(a) by Berglindh, *supra*.

Claims 6, 7, and 8 have not been rejected because, as the Examiner acknowledges, Berglindh "does not teach modifying the organic [complex] or the antigen to increase their charges." Office Action at page 4. Similarly, Popescu does not teach modifying at least one of the organic complex or the antigen to increase the degree of their respective negative or positive charge, as recited in amended claim 53.

Specifically, claim 53 recites the subject matter of claims 6 and 7: "*wherein the organic complex is modified to increase the degree of its negative charge and/or the antigen is modified to increase the degree of its positive charge.*" Applicants assert that this rejection is moot and respectfully request that it be withdrawn.

iv. Callahan would not have suggested or motivated the person of ordinary skill in the art to increase the positive charge of an antigen or the negative charge of an organic complex in an immunogenic complex, because Callahan describes only the electrostatic interactions between a malarial antigen and an aluminum hydroxide/phosphate adjuvant gel

Claims 6-8 are rejected under 35 U.S.C. § 103 over Berglindh and Cox, *supra*, further in view of Callahan *et al.*, *Pharmaceutical Research*, 8(7), pp. 851-858, 1991. According to the Examiner, Callahan teaches "the importance of surface charge in antigen-adjuvant complexes and clearly demonstrates that there is enhanced absorption between a positively charged antigen and a negatively charged adjuvant." Office Action at page 4.

(a) *Callahan only speaks of aluminum hydroxide and aluminum phosphate gels and their association with one of three malarial antigens*

Callahan does not apply to any and all adjuvants; only to the metal-based, inorganic adjuvants aluminum hydroxide and aluminum phosphate. Callahan investigates "the adsorption of three [malarial] antigens with varied isoelectric points to the commercially available adjuvants known as aluminum hydroxide and aluminum phosphate gels." Page 852,

second column, lines 9-12. Callahan emphasizes that "this work focused only on the preformed aluminum hydroxide and aluminum phosphate gels." Page 851, second column, last paragraph (emphasis added).

(b) *Callahan does not suggest that increasing the negative charge of the aluminum adjuvant and/or increasing the positive charge of the malarial antigen is desirable or even possible*

Despite this narrow focus, the Examiner alleges that the person of ordinary skill in the art would have been motivated to "augment the complementary isoelectric charges of each antigen/adjuvant component to increase antigen incorporation into the adjuvant." Office Action at page 5. There is no such motivation. Callahan emphasizes only electrostatic interactions between a malarial antigen and an aluminum-based adjuvant in a gel matrix. Callahan neither suggests nor teaches a modified aluminum adjuvant or a modified malarial antigen. That is, Callahan does not teach increasing the negative charge of the aluminum adjuvant and/or the positive charge of the malarial antigen is desirable or even possible.

(c) *Callahan is uncertain why there exists different electrostatic interactions between aluminum adjuvant and malarial antigen*

In fact, even when using the unmodified inorganic metal adjuvant and malarial antigen, Callahan is uncertain why there is greater electrostatic-mediated adsorption under certain experimental conditions than under others. Hence, Callahan admits that "the reason for the greater adsorption levels is not clear" and suggests that "structural differences, domain effects, or different adsorption mechanisms such as hydrogen bonding or hydrophobic interactions cannot be ruled out and may account for the observed results," page 856, second column, last paragraph.

Indeed, Callahan concludes that some interactions are "reversible," while others are "irreversible," and interprets certain results as applicable only "under these conditions," page 856, second column, lines 9-11. Callahan also proposes that "the mechanism of binding and the adsorbed antigen structure may be important in controlling the immune process," page 857, last paragraph.

Furthermore, nothing in Callahan suggests an *in vivo* applicability of the particular inorganic aluminum adjuvant and malarial antigen. Callahan concludes that "animal studies are needed in conjunction with additional physical characterization studies to understand further adjuvant action and to optimize immune protection," page 857, last paragraph (emphasis added).

(d) *The person of ordinary skill in the art would have thought it nonsensical to modify Berglindh's lipid adjuvant with Callahan's aluminum adjuvant*

In light of this understanding, the person of ordinary skill would have been at a loss in trying to modify Berglindh's lipid adjuvant. The skilled person would have had to have understood how to modify the lipid-based adjuvant complex, which is integral to Berglindh's composition, with Callahan's gel-based aluminum hydroxide/phosphate adjuvant.

The cited art does not teach or suggest what impact the lipid and aluminum hydroxide/phosphate adjuvants would have had on a chosen antigen. Furthermore, Berglindh does not contemplate omitting the lipid complex and Callahan does not contemplate interactions between a malarial antigen and an adjuvant other than aluminum hydroxide/phosphate. At most, the skilled person may substitute Berglindh's antigenic peptide with a malarial antigen, but that combination only reproduces a variant of Berglindh's original composition.

Accordingly, the skilled person would not have deemed the teachings of Berglindh and Callahan as fungible and would not, therefore, have ever been motivated to modify Berglindh as suggested by the Examiner.

(e) *Neither Berglindh nor Callahan suggests a "principle of increased adsorption," as posited by the Examiner*

The Examiner states that it is the "principle of increased adsorption" that would have guided the ordinary artisan's thinking at the time the time the invention was made. Office Action at page 5. That is, an increased electrostatic attraction would necessarily strengthen the association between two elements. Hence, the Examiner asserts that it would have been

desirable to increase the respective charges of antigen and adjuvant so that the association of the antigen and adjuvant is stronger.

As Applicants point out, neither Berglindh nor Callahan set forth, contemplate, or even hint at such a principle, or whether increasing the electrostatic association between adjuvant and antigen is desirable, effective, or safe.

(f) *No combination of the cited art renders claims 6, 7, or 8 unpatentable*

In summary, nothing in Callahan would have motivated the person of ordinary skill in the art to increase the negative charge of an organic complex and/or the positive charge of an antigen. Applicants contend that no combination of the cited art renders claims 6, 7, or 8 unpatentable, and respectfully request that the Examiner withdraw this rejection.

v. Conclusion

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested. The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

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CHAPTER 19

Cation-Induced Vesicle Fusion Modulated by Polymers and Proteins

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Handbook of Biological Physics
Volume 1, edited by R. Lipowsky and E. Sackmann

There are two classes of viruses in respect to the entry mechanism. Viruses with low pH-dependent activity (e.g., influenza virus, vesicular stomatitis virus) fuse with the membrane of acidic endosomes after their uptake by receptor-mediated endocytosis (fig. 1). Viruses with pH-independent activity fuse with the plasma membrane, e.g., Sendai virus, HIV (fig. 1). The pH dependence of virus fusion is a property of the viral fusion protein. The hemagglutinin of influenza virus is the best characterized fusion protein and models of the pH-dependent conformational change and penetration of a hydrophobic segment of hemagglutinin in the target membrane were given [20, 29, 30]. Synthetic peptides have been synthesized that mimic fusion regions of the viral protein and their interaction with liposomes and cells was studied [30, 31–34].

2.4. Vesicle fusion

The discussion of the natural fusion processes has shown, that the elucidation of the molecular mechanisms of membrane fusion cannot be restricted to the action of a few membrane components. Moreover, only some specific molecular components were identified to be involved in fusion processes. This specificity can result from phospholipids, glycolipids, cholesterol, membrane proteins, cytosolic proteins, metal ions such as Ca^{2+}, metabolic processes and components of the cytoskeleton. Another source of specificity is the trigger mechanism. Except the already mentioned increase of intracellular Ca^{2+} and H^+ other triggers such as changes of osmotic pressure, synthesis of unsaturated fatty acids, alterations of specific phospholipids such as phosphatidylinositol and specific proteins were discussed. The complexity of this problem requires the use of model systems which allow the separate investigation of single parts of the 'fusion machinery'.

Phospholipid vesicles and planar bilayer membranes (BLM) are the most simple model systems. Much of our current knowledge on molecular mechanisms has been obtained from studies on phospholipid vesicles. These systems have the advantage that the properties can be manipulated in a wide range (phospholipid composition, electrolyte composition, pH, incorporation of fusion effectors, size of vesicles, fluidity, nonbilayer structures). The recent success in efficiently reconstituting fusogenic proteins such as hemagglutinin of influenza virus in vesicle systems (called virosomes) shows that vesicles may serve as a valuable fundamental model for characterizing protein-membrane interactions that may lead to fusion. The potential role of calcium ions and changes in pH as triggers of membrane fusion and the modulatory role of phospholipid head groups and soluble proteins have been investigated in vesicle systems.

However, fusion requirements for vesicles are often far from those observed for biological fusion. For example, Ca^{2+}-induced fusion of vesicles occurs at much higher calcium concentrations compared with the fusion behaviour of secretory vesicles. Other limitations are (i) the lack of recognition molecules, if phospholipid vesicles are used in the simplest form and (ii) lack of postfusion stability [27]. Biological fusion processes underlay a control which removes the stimulus to fusion after the membrane fusion is completed. In vesicle systems the stimulus continues to

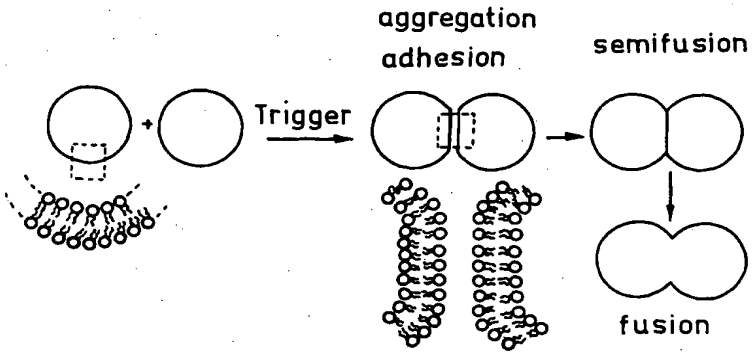

Fig. 2. Fusion reaction of two phospholipid vesicles. The first step is the aggregation and adhesion of the vesicles and the second the fusion of bilayers. In some systems, the aggregation/adhesion is followed by the semifusion (formation of one bilayer) before complete fusion with intermixing of vesicle contents occurs.

act on the vesicles after their fusion until the system reaches the final state which can considerably deviate from the state of fused vesicles. For example stacked bilayers and cochleate structures are formed in the Ca^{2+}-induced fusion of acidic vesicles, or the vesicles are transformed to the hexagonal phase [35].

It was found that the following stages occur in a fusion reaction of vesicles (fig. 2): aggregation of vesicles, molecular contact of bilayers, local destabilization of bilayers at the site of contact and intermixing of vesicle contents. Mixing of lipids of the outer monolayers can occur in the region of bilayer contact before the complete fusion, accompanied by the mixing of vesicle contents, appears. A process where the destabilization results in the formation of one bilayer between the two vesicles, causing intermixing of membrane lipids without intermixing of vesicle contents, was considered as semifusion [1].

3. Monitoring of vesicle fusion

As can be seen from figs 1 and 2 membrane fusion reactions are very complex processes. It is necessary to introduce criteria which must be met to assure that a real membrane fusion has occurred. For example, mixing of membrane components or increase in vesicle size may be the result of membrane fusion, but can also result from the exchange of membrane components between apposing membranes. It was found that the following three criteria are sufficient to define a vesicle fusion: (i) merging of membranes, (ii) intermixing of vesicle contents, and (iii) maintaining of the barrier function of membranes [27, 36]. In most vesicle fusion processes an increase in the vesicle permeability is observed due to postfusion instability as discussed before. In respect to the requirements of the fusion criteria it is important that the leakage occurs after the merging of lipids and intermixing of the aqueous contents of vesicles, even when the time delay between these processes is very short.